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This report documents results from DAMD17-97-1-7123, which is designed to identify nutritionally modulated genetic loci in the mouse that alter susceptibility to breast cancer. Calorie restriction (CR) is a potent nutritional intervention, demonstrated to reduce carcinogen induced mammary tumors in rodents. We demonstrate the modifications necessary to adapt a protocol for inducing mammary tumors with carcinogen in rats for use in mice. The study underway is currently documenting the relative mammary tumor prevalence in 8 inbred strains of mice after being dosed with carcinogen. The mice in each genotype have been divided into two dietary groups, control and CR. CR has been successfully implemented in all 8 strains after the mice were treated with carcinogen, demonstrating the feasibility of this nutritional intervention after carcinogen treatment. Among genotypes, two parameters demonstrate genotypic variability; 1) average body weight in ad libitum fed controls as a function of age and 2) caloric intake necessary to result in a 30 to 40% reduction in body weight in CR cohorts. Mammary adenocarcinomas have been observed in 5 of the 8 inbred strains being studied. Taken together, these observations suggest that this work will identify differences in response to CR among mouse genotypes.

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Ruth D. Lepow June 17, 1998.

PI - Signature Date

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INTRODUCTION

The purpose of this project is to identify nutritionally modulated genetic loci that reduce susceptibility to breast cancer. Diet is considered one of the most important environmental risk factors for cancer, estimated to contribute to 60% of the cancers in women [1]. Cognizant of the importance of diet, this project utilizes calorie restriction (CR) as the prototypical nutritional intervention affecting cancer. Our previous studies have shown that CR results in decreased prevalence of endogenous neoplastic lesions in mice [2], and the work of others has specifically indicated that CR decreases the incidence of neoplastic lesions, including breast cancer in mice and rats [3]. Although CR has been demonstrated to the delay formation, decrease the incidence and/or progression of mammary tumors of various etiologies including those induced by carcinogen, virus or mutation [4 - 6], the gene(s) controlling the effect of CR on mammary carcinogenesis have not been identified.

To identify the genetic component(s) involved in manifesting the CR response to mammary cancer causing chemicals, it is necessary to determine whether the response to CR is variable in the test species. Within the scope of this project, inbred strains of mice are being screened to determine the range of response to CR following carcinogen exposure. The desired outcome is to find mice manifesting a robust CR response and strain(s) which either show no CR response or a smaller magnitude CR response. These findings will determine the strains to use for the experiments designed to identify gene(s) responsible for differences in susceptibility to carcinogen induced breast cancer modulated by diet.

EXPERIMENTAL METHODS AND PROCEDURES

The preliminary component of this project was development of a simple, reproducible means of obtaining a high incidence of breast cancer in mice following carcinogen treatment. 7,12-dimethylbenz[a]anthracene (DMBA) is a polycyclic aromatic hydrocarbon which is a metabolism dependent procarcinogen [7] and in rodents is a potent mammary carcinogen [8]. As reported by several investigators [9,10] a single oral gavage of DMBA induces mammary tumors in rats. This method of carcinogen induced breast cancer was selected as the method of choice for this work because it minimized the handling of and stress to the animals.

The initial experiment conducted as part of this project was to determine what modifications would be necessary to adapt the published protocol for inducing mammary cancer in rats with a single dose of DMBA, for use in mice. For this experiment, 9 week old female C3H/HeNHsd mice were dosed with 65 mg DMBA / kg body weight as reported [9,10] via oral gavage. After dosing, each mouse was placed in a metabolic cage to facilitate collection of DMBA contaminated excreta, and the animals were observed daily for clinical signs of disease. Although no difficulties were experienced gavaging the mice and they were all observed to be active within minutes of dosing, a number of mice were lost within several days of dosing. These animals appeared lethargic and manifested clinical signs of dehydration. Affected animals were given a subcutaneous (SQ) injection of saline and were provided with an additional source of water. Despite these interventions, the affected mice died. A second cohort of 9 week old female C3H/HeNHsd mice were dosed with 65 mg DMBA / kg body weight and given an SQ injection of saline following DMBA dosing, as well as being given an additional source of water in their cages. A significant difference in mortality was observed comparing mice which did and did not receive hydration support immediately following DMBA administration (Figure 1). Appendix 1 contains a manuscript detailing the enhanced survival during the first six weeks

after DMBA dosing through the use of prophylactic hydration. This manuscript has been submitted for publication.

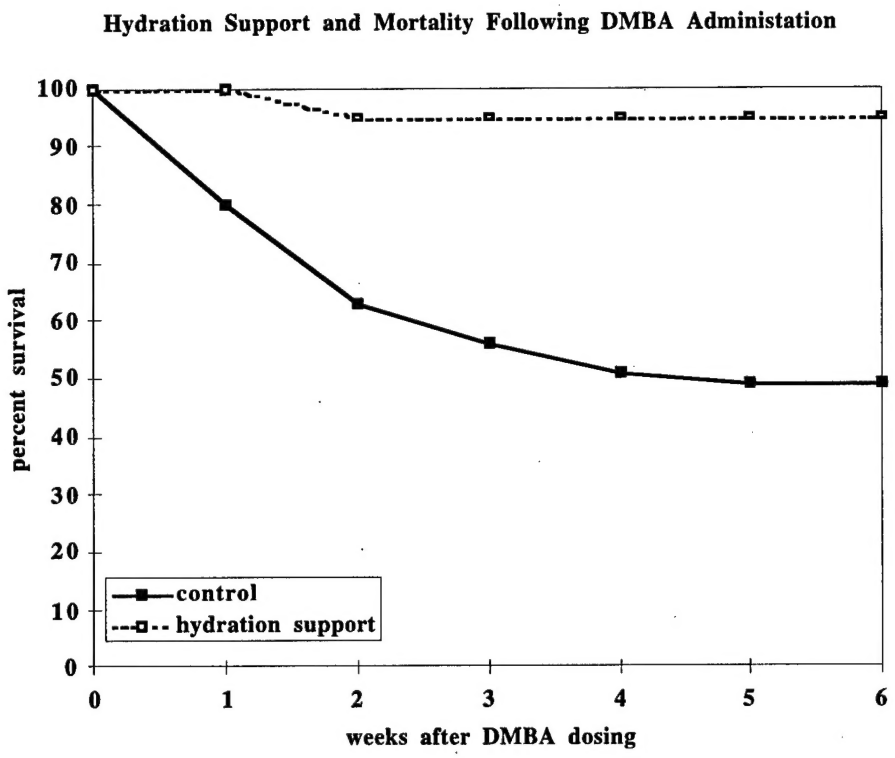

Figure 1: Comparison of acute mortality following dosing with DMBA between groups receiving prophylactic hydration support and those receiving hydration support after exhibiting symptoms of dehydration.

Our initial experience, with a substantial percent of mice succumbing after DMBA dosing without prophylactic hydration support, raised the question of whether the proposed experiment would be plagued with differential acute sensitivity to the carcinogen among the various strains to be used. Initial work with 8 inbred strains of mice demonstrated that with prophylactic hydration support, acute toxicity, as defined as death within 4 weeks of DMBA administration, is low and there are no significant difference among the strains tested (Figure 2).

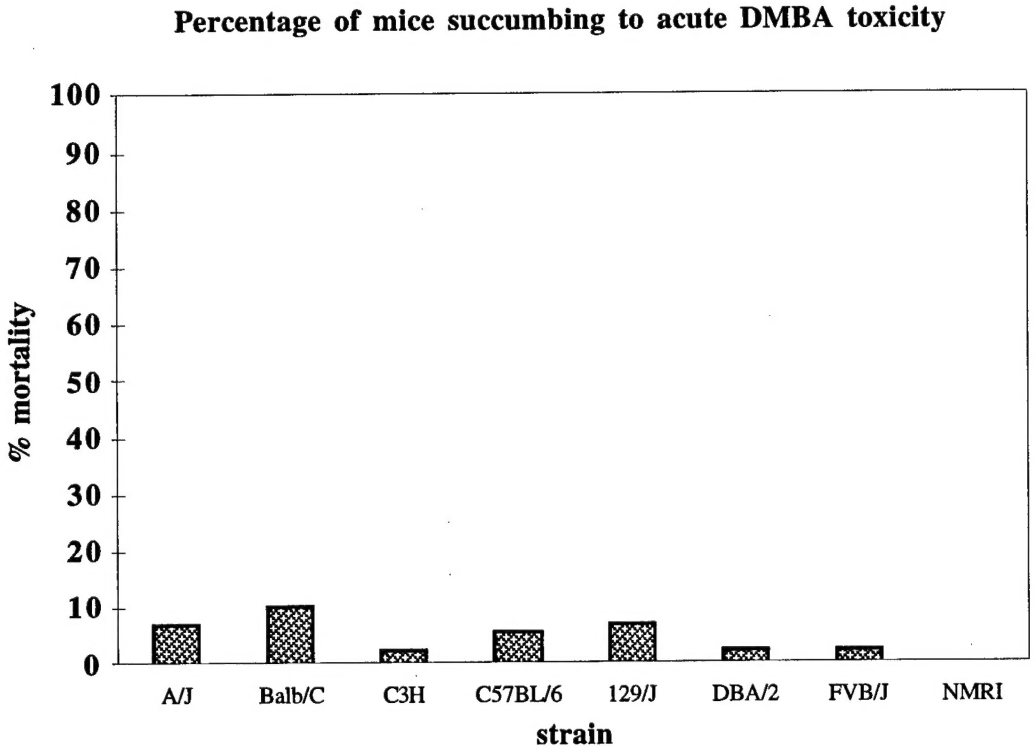

Figure 2: The acute mortality following DMBA administration is low and does not demonstrate significant differences among the mouse genotypes examined using an experimental protocol which incorporates the use of prophylactic hydration support.

The next critical determinant in the feasibility of the proposed experiment was to establish whether the severity of the immuno-suppressive effects of DMBA would compromise the study. A total of 21 mice (7%) have succumbed, thus far, to what appear to be infections. Among the 6 strains for which deaths due to infection have been observed, the prevalence of fatal infections during this period differed among the strains ($p \leq 0.04$) with the Balb/C mice having a greater apparent susceptibility than the other strains. This increased susceptibility in the Balb/C mice was observed in both control and CR diet groups (Figure 3). Within the Balb/C strain, there were a comparable number of fatalities with infections in the control and CR groups. This may suggest that further modification to the dosing regime or the number of animals utilized to facilitate study of the Balb/C strain may be needed. For the other 7 strains currently under study, the conditions of DMBA dosing appear

suitable for following the animals to study the degree to which CR will ameliorate mammary lesions.

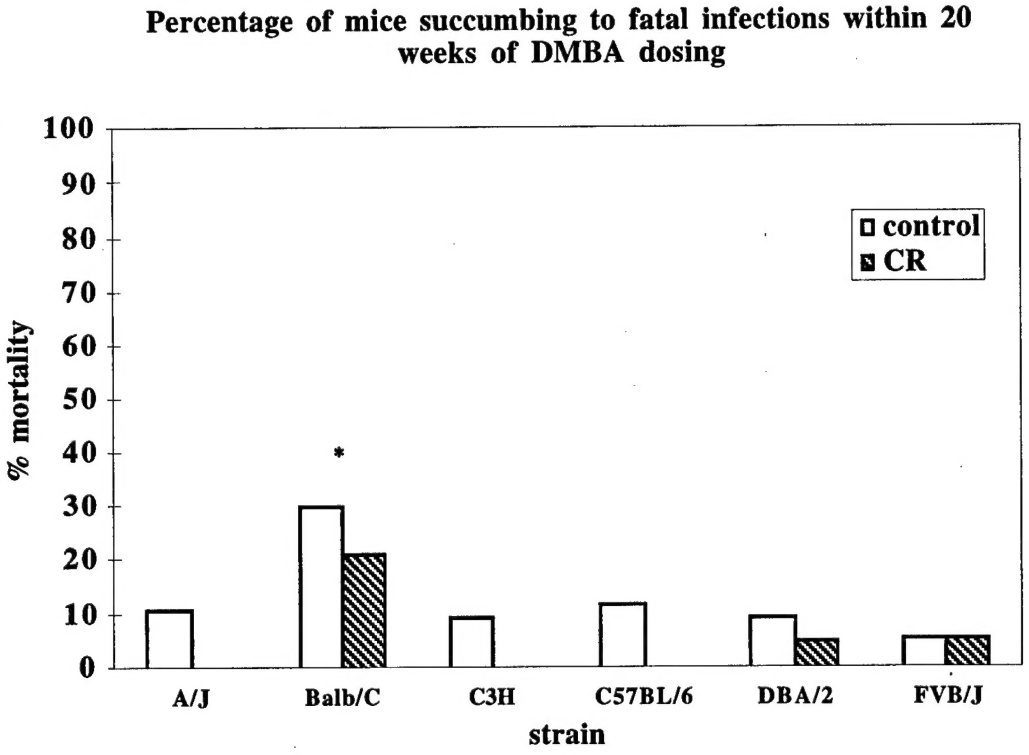

Figure 3: The mice were followed in the post dosing period to determine whether the immuno-suppressive effects of DMBA would either be so severe as to compromise the study as designed or whether there would be differential susceptibility among the genotypes. Increased susceptibility to these effects was observed for the Balb/C mice, independent of diet ($p \leq 0.05$).

The experiment to establish the range of responsiveness to CR is currently underway. Dosing of the various inbred strains of mice was staggered thus making comparison of responses of all 8 genotypes possible only up to 12 weeks after DMBA administration. This time period is too soon after dosing to expect substantial numbers of mammary cancer cases. The number of deaths within each genotype are, to date, too small to discern diet based differences in mortality. By combining the mortality data from all genotypes, there is enhanced survival of the CR cohort compared with the ad libitum fed

controls ($p \leq 0.004$). This analysis, however, does not factor in the different lengths of time from DMBA dosing for the 8 genotypes of mice currently under study.

At this point in time, there are approximately 333 mice that have been dosed with DMBA as part of this study. The elapsed time since DMBA dosing ranges from 12 to 34 weeks. As seen in Figure 4, cases of proliferative mammary lesions are beginning to be observed.

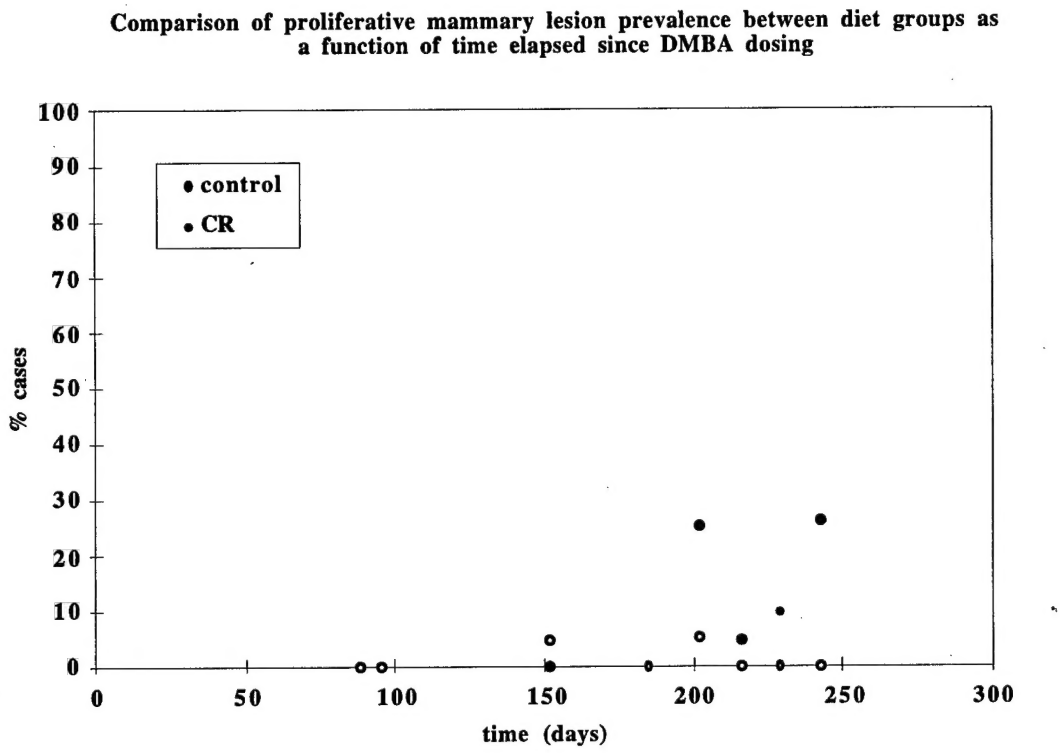

Figure 4: Observation of proliferative mammary lesions is dependent in part, to the length of time which has elapsed since DMBA dosing.

Preliminary results show the range of response to CR in terms of decreased percentage of mammary lesions for those genotypes in which mammary lesions have been observed in the control cohorts ranges from a 5% decrease in the DBA/2 to a 26% decrease in the Balb/C. As the time elapsed from DMBA dosing increases, it is anticipated that the percentage of mice observed with mammary lesions will continue to increase.

We have expanded our definition of CR response to include its effects on the prevalence of all neoplastic lesions. We have observed responsive CR ranges from a 5% reduction in the the FVB/J to a 40% reduction in neoplastic lesions prevalence in the Balb/C (Figure 5).

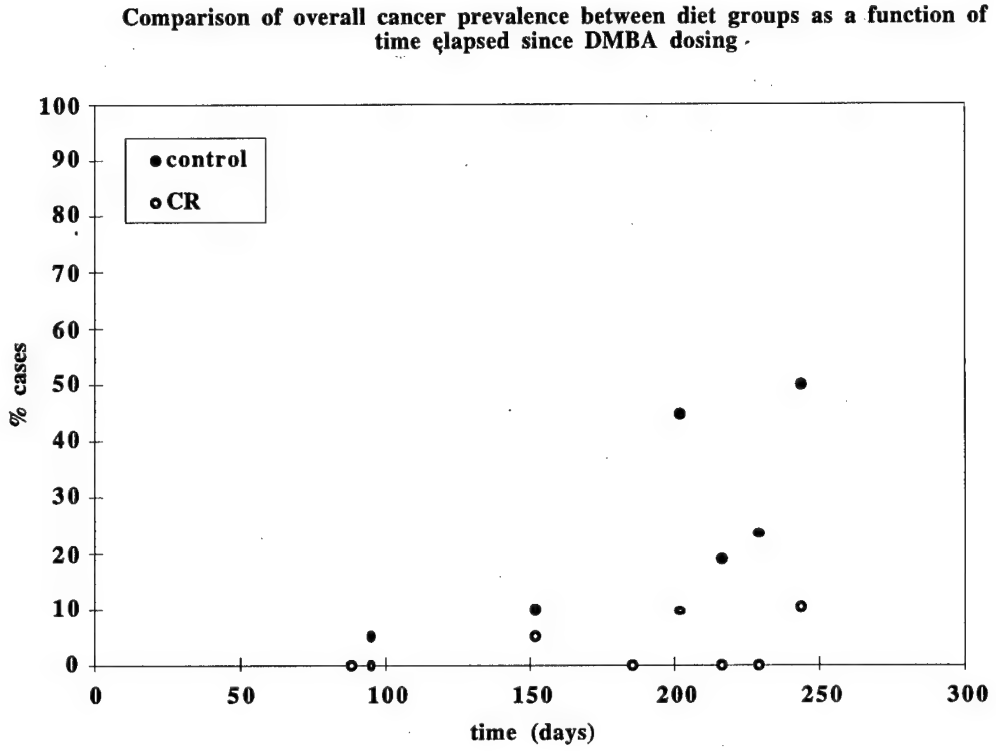

Figure 5: Prevalence of proliferative lesions is, in part, related to the time which has elapsed since DMBA dosing.

It is still too early in the experiment to draw conclusions regarding our ability to discern differential response of the various genotypes to CR. Figure 6, in presenting the relative survival among the genotypes for the both diet groups, shows that most of the genotypes currently under study have yet to attain 50% mortality.

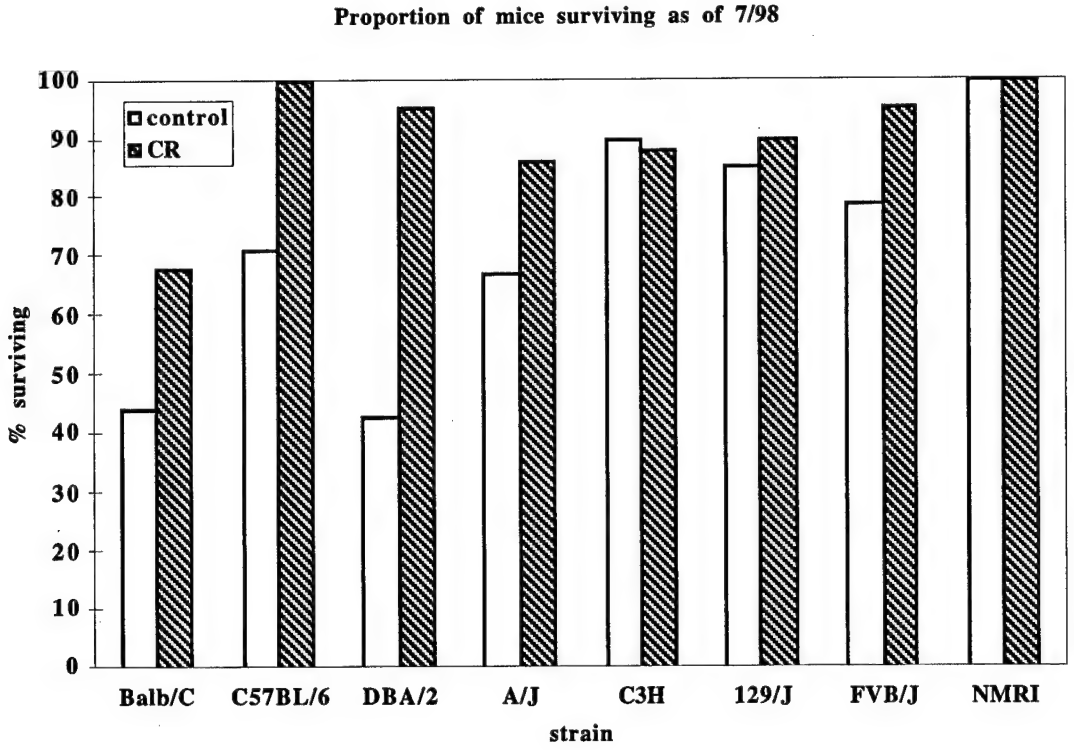

Figure 6: Mortality kinetics reflects, in part, the range in time elapsed since the mice in each genotype were dosed with DMBA.

ASSUMPTIONS

Successful execution of the proposed experiments were predicated on several assumptions. The first was the tacit assumption that a methodology for carcinogen induced mammary cancer in rats would result in a similar prevalence of mammary cancer in mice. Concomitant with this assumption, is that the timing for carcinogenisis would be the same for the two species. It will be necessary to wait for sufficient time to elapse to make determinations regarding both of these.

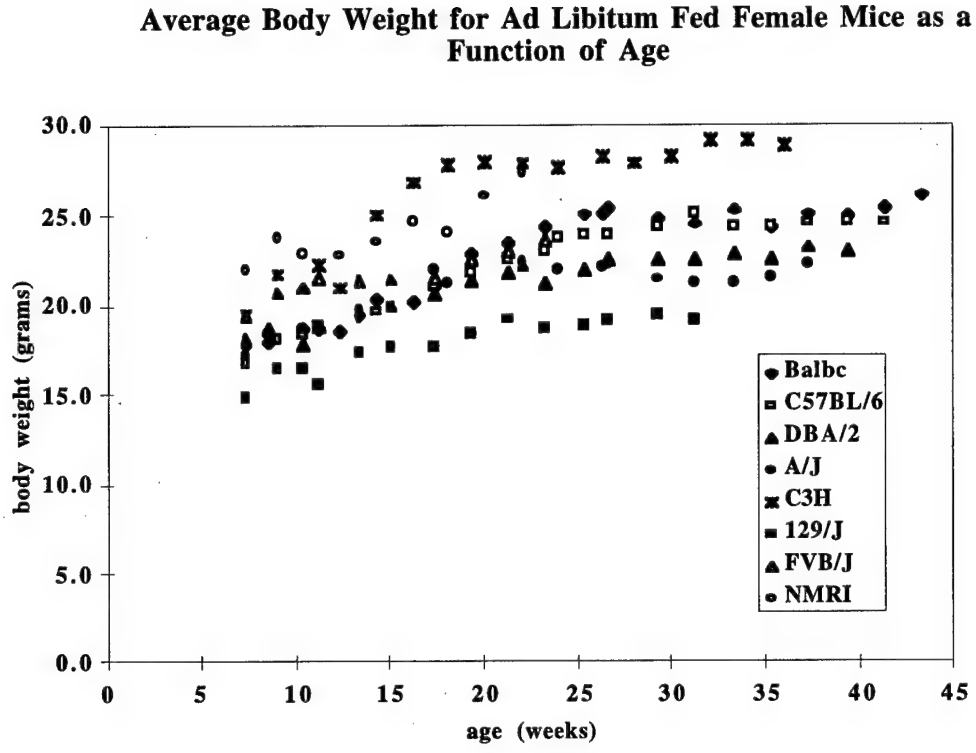

Figure 7: Variability in average body weight among for the ad libitum fed cohorts of the 8 genotypes as a function of the age. All the data is from mice which had been dosed with DMBA.

This experimental paradigm is also predicated on the assumption that within inbred strains, there are differential responses to CR such that there would be strains in which the prevalence of carcinogen induced mammary cancer would be greatly reduced and strain(s) in which the prevalence of carcinogen induced mammary cancer would be reduced to a much smaller extent. Figure 7 demonstrates that there is variability in the body weights for the ad libitum fed cohorts. This can be taken as evidence that there is sufficient genetic difference among these genotypes that there may be differences in their responsiveness to CR.

It was also assumed that with all genotypes of mice, it would be possible to restrict caloric intake after DMBA dosing without fatal consequences. Figure 8 demonstrates that we have successfully initiated CR in all genotypes and as was demonstrated in Figure 6, to date, there has not been increased incidence of death in the CR cohorts.

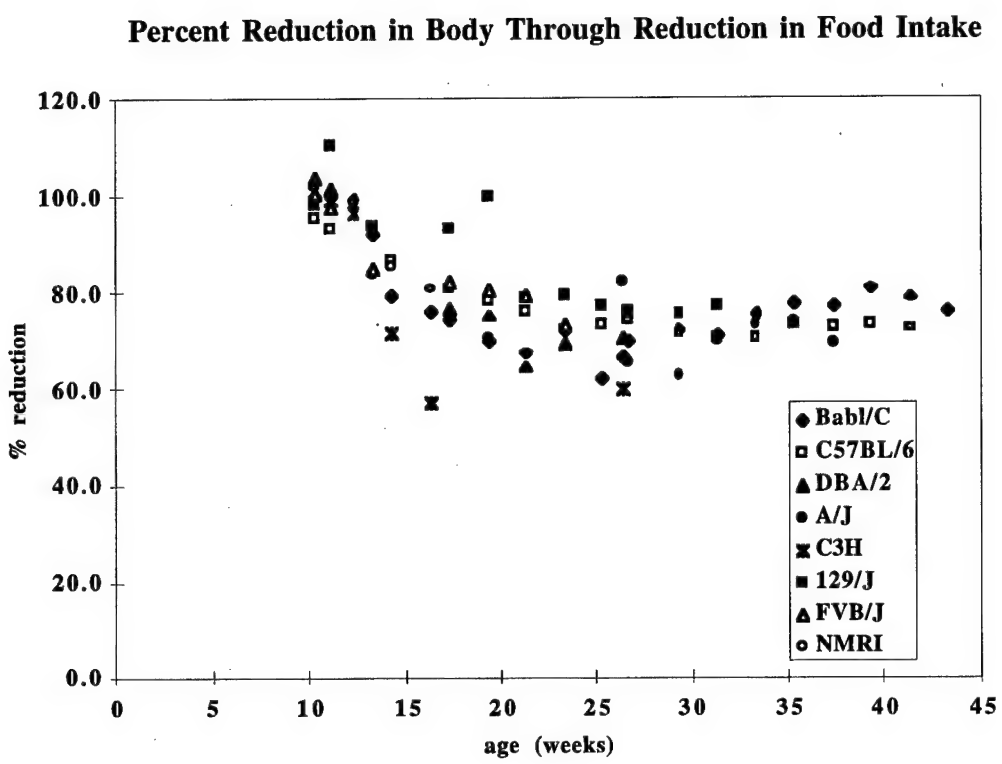

Figure 8: It has been possible, following DMBA dosing, to decrease the caloric intake of mice in all genotypes studied. With the introduction of CR, there has been a concomitant decrease in weight for all genotypes.

RESULTS

Our results to date, include our finding of increased sensitivity to dehydration exhibited by mice as compared to rats when given equivalent doses of DMBA based on body weight. This finding may have application to other studies in mice involving perturbations other than DMBA administration.

Another clear finding from this study is the extent to which average body weight of inbred strains of mice varies (Figure 7). This has impacted on the current experimentation in several ways. The dose of DMBA ranged from 0. 21 to 0.29 cc per mouse in order to deliver comparable doses of DMBA to all genotypes on a per body weight basis. Not surprisingly, it has also been necessary to titrate the food intake for each strain individually in order to obtain reductions of body weight for the CR cohorts of 30-40% (Figure 8).

Given the ranges in food consumption and average body weights, it appears likely that ranges in response to CR will be observed.

CONCLUSIONS

The experimentation for this project is a work in progress. We have found that mice exhibit increased acute sensitivity to DMBA dosing than that reported for rats. This increased sensitivity can be ameliorated through the use of prophylactic hydration support. Our study demonstrates that CR can be initiated in mice which have been dosed with DMBA. We now believe that mammary cancer develop in the mouse after DMBA administration may take somewhat longer than in the rat. However, we conclude that the mouse is a suitable species in which to study DMBA induced mammary cancer as we have observed cases of well circumscribed nodular masses with histologic growth patterns consistent with mammary adenocarcinoma in 5 inbred strains to date.

REFERENCES

1. Doll, R. and Peto, R. The cause of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. J. Natl. Cancer Inst. 1981;66: 1191-1308 .

2. Bronson, R.T. and Lipman, R.D. Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. Growth, Dev. Aging. 1991;55: 169-184 .

3. Weindruch, R. and Walford, R.L., *The Retardation of Aging and Disease by Dietary Restriction*. 1988, Springfield, IL: C.C. Thomas.

4. Klurfeld, D.M., Welch, C.B., Lloyd, L.M., and Kritchevsky, D. Inhibition of DMBA-induced mammary tumorigenesis by caloric restriction in rats fed high-fat diets. Int. J. Cancer. 1989;43: 922-925 .

5. Engelman, R.W., Day, N.K., and Good, R.A. Calories, parity, and prolactin influence mammary epithelial kinetics and differentiation and alter mouse mammary tumor risk. Cancer Res. 1993;53: 1188-1194 .

6. Li, H.W., Zhao, W., and Sarker, N.H. Dietary regulation of mammary tumorigenesis in RIII/Sa mice: investigation of a possible mechanism. Cancer Letters. 1994;79: 199-211 .

7. Brookes, P. and Lawley, P.D. Evidence for the binding of polynuclear aromatic hydrocarbons to the nuclei acids of mouse skin: relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid. Nature. 1964;202: 781-784 .

8. Lau, H.H., Coffing, S.L., Lee, H., Harvey, R.G., and Baird, W.J. Stereoselectivity of activation of 7,12-dimethylbenz[a]anthracene-3,4-dihydrodiol to the anti-diol epoxide metabolite in a human mammary carcinoma MCF-7 cell-mediated V79 cell mutation assay. Chem. Res. Tox. 1995;8: 970-978 .

9. Haag, J.D., Newton, M.A., and Gould, M.N. Mammary carcinoma suppresser and susceptibility genes in the Wistar-Kyoto rat. Carcinogen. 1992;13: 1933-1935 .

10. Hsu, L.-C., Kennan, W.S., Shepel, L.A., Jacob, H.J., Szpirer, C., Szpirer, J., Lander, E.S., and Gould, M.N. Genetic identification of Mcs-1, a rat mammary carcinoma suppresser gene. Cancer Res. 1994;54: 2765-2770 .

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Improved Survival Rates in Mice with Prophylactic Fluids Following Carcinogen Treatment

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Abstract

During the development of a model for 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary adenocarcinoma in mice, a high mortality rate was attributed to dehydration. Thus, the acute survival of mice given subcutaneous fluids prophylactically immediately following DMBA gavage were compared to those provided treatment only when clinical signs of dehydration were observed. Mortality in the prophylactically treated mice was 5% compared to 47% in animals treated after the manifestation of dehydration. Prophylaxis with subcutaneous fluids significantly reduces mortality in DMBA-treated mice.

Introduction

Experimental protocols based on one animal model may require modification when applied to a different animal due to species differences in physiology. We developed a breast cancer model in mice based upon previous work in rats utilizing the pro-carcinogen 7,12-dimethylbenz[a]-anthracene (DMBA) (1,2) which forms depurinating DNA adducts in rat mammary epithelial cells (3). DMBA use as a carcinogen in rodents is common with a variety of doses, number, and routes of administrations reported (1,2,4-7). We selected the DMBA method described by Haag et al. (2) and Hsu et al. (1) because it appeared to allow for a smaller sample size and minimal stress to the animals (8). This method is reported to result in a 100% incidence of mammary adenocarcinoma in susceptible rat genotypes without early mortality (2,1). The protocol involves only a single administration of carcinogen and thus minimizes both stress to the animals and the potential for environmental

contamination. The modifications necessary to manage the hydration status of DMBA-treated mice and minimize acute mortality are described.

Material and Methods

This study was approved by the USDA Human Nutrition Research Center on Aging Animal Care and Use Committee. Two cohorts of 20 six wk old female C3H/HeNHsd mice (Harlan Sprague Dawley, Indianapolis, IN) were individually housed in 8" x 8" x 8" suspended, polycarbonate cages and provided *ad libitum* access to NIH31 diet (Harlan Teklad, Madison, WI) and purified water sterilized by UV irradiation. The mice were acclimated to appropriate environmental conditions for 3 wk prior to carcinogen exposure (9,10). At this time, all animals were observed daily for clinical signs of disease and weighed each week.

Working within a fume hood, DMBA (Sigma Chemical, St. Louis, MO) was dissolved in sesame seed oil (Sigma Chemical) to a concentration of 5.2 mg/mL. The first cohort of 20 mice were anesthetized within a negative pressure hood with Aerrane® Isoflurane, USP (Fort Dodge Animal Health, Fort Dodge, IA) and orally gavaged with 0.13 mL DMBA to provide 65 mg DMBA/kg body weight. The second cohort were similarly dosed but prior to recovery from anesthesia each mouse was injected subcutaneously (SQ) with 1.0 mL 0.9% NaCl (Abbott Laboratories, North Chicago, IL). In addition to the water bottle with sipper tube present in each metabolic cage, this second cohort of mice were also given a jar of drinking water.

Three days after dosing, 80% of a DMBA dose is reportedly present in the excreta (11) and no biologically active carcinogen remains *in vivo* 5 d after an oral

gavage (12). Accordingly, mice were housed in metabolic cages (Lab Products, Maywood, NJ) for 1 wk to facilitate collection of all feces and urine potentially contaminated with DMBA. A plastic bag was used to enclose the entire urine/feces separation unit so as to minimize potential carcinogen contamination of the area. All excreta were disposed of as chemical waste. Personnel safety procedures including protective face shield and disposable garb were used as previously described (13). Access to the animal room (maintained at negative pressure) was restricted.

Comparison of mortality incidence between groups was carried out with a 2 x 2 χ^2 analysis. Average body weights of the mice were compared using a two-tailed t-test. Statistical analyses were conducted with Statools (14).

Results

No difficulties were experienced gavaging the mice and all were observed to be ambulatory and active upon recovery from anesthesia within 1-2 min. All mice appeared to be in a similar condition 24 h after dosing. Forty eight - 72 h after DMBA dosing, 3 animals in the first cohort were lethargic with clinical signs of dehydration, including anorexia, anuria, loss of skin elasticity, and skin turgor. Mice observed with these clinical signs were given a 1.0 mL saline SQ and a water jar was placed in their cage. Despite this supportive fluid therapy, the condition of these mice did not improve and they died within 48 h. Eight additional mice in this cohort were observed with similar clinical signs up to 4 wk after DMBA treatment and were also provided with supportive hydration; these mice too subsequently

died within 48 h.

The difference in post-dosing mortality was significantly different ($p \leq 0.05$) between groups (Fig. 1). The cumulative 4 wk post-procedure mortality for the first cohort of mice was 47%. This compared with a loss of only 5% (one mouse) during the same 4 wk period after dosing for mice receiving the prophylactic injection of saline immediately following DMBA dosing. The average weight two weeks following dosing of the mice in the first cohort (18.6 ± 1.51 g) which went on to die in the next two weeks was significantly less than the prophylactically treated mice two weeks after dosing (22.3 ± 2.3 g) ($p \leq 0.005$). The average weight of the surviving mice 4 weeks after DMBA administration did not differ between the first cohort and the prophylactically treated mice (21.3 ± 1.8 v. 22.3 ± 2.3 g).

Discussion

The induction of mammary tumors in the rat with the use of chemical carcinogens is a commonly utilized model for the study of breast cancer (15). While the rat model is ideal for some experiments, there are valid reasons for examining phenomena in other species including the facilitation of specific analyses or the comparison of effects between species. We highlight here the critical importance of prophylactic hydration to survival of mice treated with DMBA. Basic veterinary care, i.e., provision of supportive fluid therapy upon presentation of clinical signs, was insufficient to prevent the high mortality associated with the effective DMBA dose. The timing of fluid administration as a supportive measure is an important factor and may be narrowly defined in rodents (16). Prophylactic interventions to

facilitate hydration may be prudent for the adaptation of other rat protocols to mice as a variety of significant physiologic functions including immune responses, renal cortical blood flow, and drug distribution are altered by hydration status in mice (17-19). Subcutaneous fluid administration as a means of rehydration has also been demonstrated to be effective in other species, including humans (20). As suggested as well by Dieterich et al. (17), this study reinforces the necessity of performing small scale pilot studies to adapt published protocols from one animal model to another prior to initiating large experiments.

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References

1. **Hsu, L.-C., Kennan, W.S., Shepel, L.A., Jacob, H.J., Szpirer, C., Szpirer, J., Lander, E.S., and Gould, M.N.** 1994 Genetic identification of Mcs-1, a rat mammary carcinoma suppressor gene. Cancer Res. **54**: 2765-2770.

2. **Haag, J.D., Newton, M.A., and Gould, M.N.** 1992 Mammary carcinoma suppressor and susceptibility genes in the Wistar-Kyoto rat. Carcinogen. **13**: 1933-1935.

3. **Todorovic, R., Ariese, F., Devanesan, P., Jankowiak, R., Small, G.J., Rogan, E., and Cavalieri, E.** 1997 Determination of beno[a]pyrene- and 7,12-diemthylbenz[a]anthracene- DNA adducts formed in rat mammary glands. Chem. Res. Tox. **10**: 941-947.

4. **Klurfeld, D.M., Welch, C.B., Lloyd, L.M., and Kritchevsky, D.** 1989 Inhibition of DMBA-induced mammary tumorigenesis by caloric restriction in rats fed high-fat diets. Int. J. Cancer. **43**: 922-925.

5. **Qing, W.G., Conti, C.T., LaBate, M., Johnston, D., Slaga, T.J., and MacLeod, M.C.** 1997 Induction of mammary cancer and lymphoma by multiple, low oral doses of 7,12-dimethylbenz[a]anthracene in Sencar mice. Carcinogen. **18**: 553-559.

6. **Witty, J.P., Lempka, T., Coffey, R.J., and Matrisian, L.M.** 1996 Decreased tumor formation in 7,12-dimethylbenzyanthracene-treated Stromelysin-1 transgenic mice is associated with alterations in mammary epithelial cell apoptosis. Cancer Res. **55**: 1401-1406.

7. **Terada, S., Uchide, K., Suzuki, N., Akasofu, K., and Nishida, E.** 1995 Induction of ductal carcinomas by intraductal administration of 7,12-dimethylbenz[a]anthracene in Wistar rats. Breast Cancer. Res. Treat. **34**: 35-43.

8. **Rao, G.N. and Huff, J.** 1990 Refinement of long-term toxicity and carcinogenesis studies. Fund. Appl. Tox. **15**: 22-43.

9. **Lipman, R.D. and Grinker, J.A.** 1995 Weight gain by middle-aged mice: dietary modification does not result in loss. Growth Dev. Aging. **60**: 61-70.

10. **Lipman, R.D., Bronson, R.T., Wu, D., Smith D.E., Prior, R., Cao, G., Han, S.N., Martin, K.R., Meydani, S.N., Meydani, M.** 1998 Disease incidence and longevity are unaltered by dietary antioxidant supplementation initiated during middle age in C57BL/6 mice. Mech. Ageing Dev., in press.

11. **Clinton, S.K. and Visek, W.J.** 1989 Hepatic and intestinal drug-metabolizing enzymes and the tissue distribution and excretion of ^{14}C-7,12-dimethylbenz[a]anthracene in rats fed diets varying in fat content. Pharm. **39**: 89-97.

12. **Archuleta, M.M., Born, J.L., Montano, R.M., and Burchiel, S.W.** 1992 Covalent binding of 7,12-dimethylbenz[a]anthracene to lymphoid and nonlymphoid tissues following oral administration to B6C3F1 mice. Tox. App. Pharm. **113**: 133-137.

13. **Smith, D., Morrow, R., and Patnaude, D.** 1993 A program for safe carcinogen use in rodents. CALAS/ACTAL. **27**: 5-9.

14. **Dallal, G.E.** 1986 STATOOLS: Statistical utility programs. Am. Statistician. **40**: 236.

15. **Russo, J. and Russo, I.H.** 1996 Experimentally induced mammary tumors in rats. Breast Ca. Res. Treat. **39**: 7-20.

16. **Lundblad, R. and Gierchsky, K.E.** 1995 Synergistic effects of E5, imipenem, and volume support during fulminant intraabdominal sepsis in rats. J. Inf. Dis. **172**: 152-160.

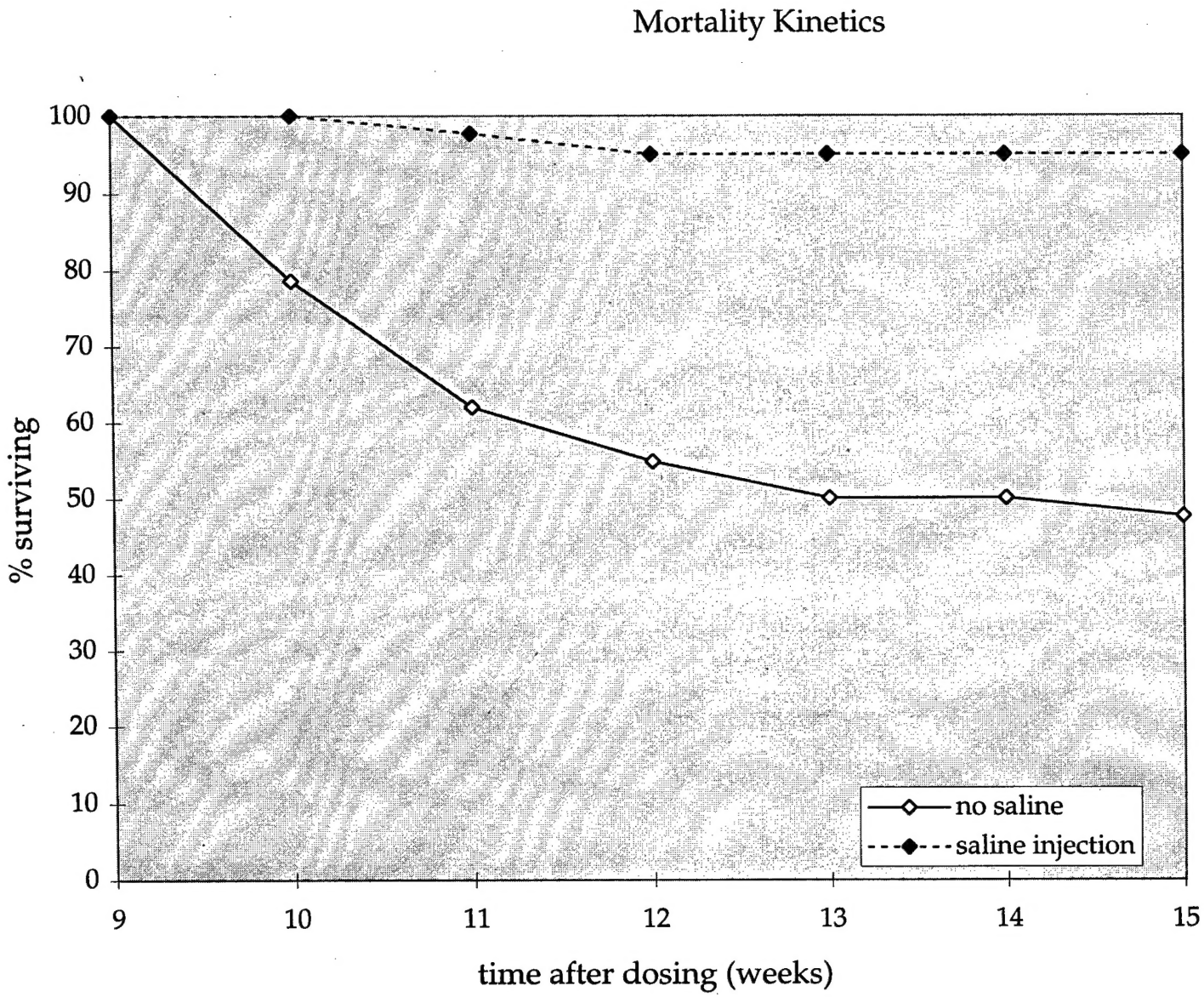
17. **Dieterich, D.T., Poles, M.A., Lew, E.A., Martin-Munely, S., Johnson, J., Nix, D., and Faust, M.J.** 1997 Treatment of gastrointestinal cytomegalovirus infection with twice-daily foscaret: a pilot study of safety, efficacy, and pharmokinetics in patients with AIDS. Antimicro. Agents Chemo. **41**: 1226-1230.

18. **Hogstrom, B., Hietala, S.O., and Rooth, P.** 1996 Effects of mannitol and iohexol infusions on the renal cortical blood flow in dehydrated mice. Acta. Rad. **37**: 591-595.

19. **Cronhjort, M., Schnell, P.O., and Jacobsson, H.** 1994 Disturbances of fluid balance reduce the image quality of bone scintigraphy. Experimental studies in mice. Nuc. Med. Comm. **15**: 469-474.

20. **Nobel-Adams, R.** 1995 Dehydration: subcutaneous fluid administration. Brit. J. Nutr. **4**: 488-494.

Figure 1


Mortality Kinetics
% surviving
100
90
80
70
60
50
40
30
20
10
0
9
10
11
12
13
14
15
time after dosing (weeks)
no saline
saline injection